Recovery of Salmonellae from Trisodium Phosphate-Treated Commercially Processed Broiler Carcasses After Chilling and After Seven-Day Storage

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ABSTRACT Experiments were conducted to determine the effect of prechill trisodium phosphate (TSP) treatment on reducing salmonellae recovery from broiler carcasses immediately after chilling or following 7 d of storage. Carcasses were sampled for salmonellae using whole carcass enrichment for 24 h at 37°C. In each of 7 trials, 40 carcasses were obtained from a commercial processing plant. Batches of 4 carcasses were subjected to a 5-s dip in 10% TSP (treatment) or not dipped (control). Two carcasses from each batch were sampled immediately after chilling (d 0) and 2 carcasses were sampled after 7 d of storage. For trials 1 and 2, TSP treatment and control groups were chilled in separate chill tanks for 45 min. For trials 3 through 7, carcasses were rinsed with water and individually bagged with ice and water before chilling. For trials 1 and 2, 85% (17/20) of control carcasses were salmonellae-positive on d 0 compared with 45% (9/20) of the TSP-treated carcasses; after 7 d, 75% (15/20) of control carcasses were positive compared with 35% (7/20) for the TSP-treated carcasses. For trials 3 through 7, 46% (23/50) of control carcasses were salmonellae-positive on d 0 compared with 26% (13/50) of the TSP-treated carcasses; after 7 d, 20% (10/50) of control carcasses were positive compared with 4% (2/50) of the TSP-treated carcasses. TSP treatment resulted in significantly higher pH values for rinses. Salmonella recovery was decreased by refrigerated storage and treatment with TSP before immersion chilling.

(*Key words*: broiler, chilling, salmonellae, trisodium phosphate, whole carcass enrichment)

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INTRODUCTION

Trisodium phosphate (TSP) has been shown to reduce the recovery of salmonellae from processed poultry (Giese, 1992; Kim et al., 1994a,b; Li et al., 1994; Lillard, 1994; Somers et al., 1994). The use of TSP as a prechill carcass wash was patented by Bender and Brotsky (1991), and has been approved for the reduction of *Salmonella* in poultry processing plants by the USDA (Giese, 1993). According to the Food Safety and Inspection Service (2003), 11.5% of broilers sampled from US processing plants in 2002 were positive for *Salmonella*.

The decrease in salmonellae recovery from poultry carcasses immediately after treatment with TSP has been reported previously (Hwang and Beuchat, 1995; Colin and Salvat, 1996; Ellerbroek et al., 1996; Rodriguez de Ledesma et al., 1996). However, the effectiveness of TSP on salmonellae recovery at the retail level (5 to 7 d post-processing) is less clear. Residual effects of TSP on *Salmonella* recovery (0.5 log reduction) after refrigerated storage for 6 d have been reported (Kim et al., 1994b). In these experiments, excess TSP was not rinsed off before placing the carcass in buffered peptone water for sampling. However, in a commercial application, TSP would be rinsed off the carcass during immersion chilling before salmonellae sampling.

In most US processing plants, carcasses treated with TSP drip for about 1 min before being dropped into (without rinsing) an immersion chiller of water and ice maintained below 40°C for 30 to 90 min. Residual TSP on the carcasses is washed off during immersion chilling, raising the chiller water pH. Elevated pH has been shown to decrease chlorine efficiency in the chiller and chill water is often neutralized to optimize chlorine effectiveness.

The objective of the current study was to determine the efficacy of TSP on day of processing and after 7 d of storage at 2°C. Evaluation at 7 d was to determine whether TSP would affect salmonellae recovery on products as purchased by the consumer.

MATERIALS AND METHODS

Carcass Treatment

On each of 7 trial days, 40 broiler carcasses were obtained by removing them from the shackle line of a com-

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Abbreviation Key: TSP = trisodium phosphate.

mercial processing plant immediately following the inside-outside bird washer, and before TSP treatment and chilling. Each carcass was individually placed in a clean plastic bag and transported to the pilot processing plant.

At 15 min intervals, groups of 4 carcasses were identified with wing bands and subjected to an inside-outside bird washer[2] at 551.6 kPa (80 psi) for 10 s on shackles spaced every 30.5 cm (12 in). This second inside-outside carcass wash was done to rewet the carcass as it would have been in the commercial processing plant prior to TSP treatment. Tap water with no added chlorine was used to limit the decrease of salmonellae due to an additional chlorine wash. Alternating groups of 4 carcasses were submerged for 5 s in a 10% TSP solution at 24°C or were nontreated controls. TSP-treated carcasses were allowed to drip for 1 min to simulate plant exposure time before immersion chilling. Control carcasses were placed directly into chiller tanks. In a typical commercial situation, carcasses are either treated with TSP or go directly into the chiller without an additional water-dipping step, and this process was followed for control carcasses in this trial.

Immersion Chilling Method

After treatment, carcasses in trials 1 and 2 were chilled in pilot scale immersion paddle chill tanks (228 L) filled with water and ice. One chill tank was used for TSP-treated carcasses and the other for control carcasses. Successive batches were added without emptying the chillers. All carcasses were chilled for 45 min with an approximate water overflow rate of 1 L/min to diminish chill water suspended solids and TSP concentration during the chill period (simulating commercial chilling). Chiller water pH was recorded at the end of trial 2. Due to higher chill water pH values in the TSP chiller, compared with the control chiller, the chilling procedure was modified.

Individual Bag Chilling Method

To reduce the possibility of salmonellae cross-contamination and to better control chill water pH during chilling, an individual carcass bag chilling method was developed. Following treatment, carcasses in trials 3 through 7 were thoroughly rinsed off inside and outside with a hose 4 times by hand to minimize residual TSP before entering the chill tank. Carcasses were immediately placed into clean plastic bags (36 × 51 cm). To each bag (control and TSP-treated carcasses), 300 mL of 20× PBS (1× = 1.47 m*M* KH_2PO_4, 10 m*M* Na_2HPO_4, 2.7 m*M* KCl, 137 m*M* NaCl, pH = 7.4), 1.7 L of tap water, and 2 L of crushed ice were added. Phosphate-buffered saline was added to reduce and buffer the pH increase caused by TSP. Excess air was expelled and bags sealed with a cable tie. Bagged carcasses were placed into an immersion paddle chill tank filled with ice and water. Following 20 min in the chill tank, each bagged carcass was taken out of the ice water, aseptically removed from the bag, and placed into a second bag. Two liters of tap water and 2 L of ice without PBS were added and the bag sealed and chilled for an additional 20 min in a second chiller. The pH was recorded from the chill water in each bag after the first and second chill. The pH of the chill tank water was measured before and after chilling to determine possible bag leakage.

Salmonellae Sampling

After chilling, carcasses were hung on sanitized shackles, allowed to drip for 5 min, placed into individual polyethylene bags (41 × 41 cm), and separated into 2 sampling groups. One group of 20 carcasses was sampled on the day of processing and the other group (n = 20) was sampled following 7 d of storage at 2°C. Each carcass was sampled for salmonellae using whole carcass enrichment similar to the method described by Simmons et al. (2003). Carcasses were shaken for 1 min in 500 mL of buffered peptone water,[3] and incubated in the rinse solution for 24 h at 37°C. After incubation, 0.1 mL of the rinse solution was transferred to Rappaport-Vassiliadis broth[3] and 0.5 mL of the rinse solution was transferred to tetrathionate broth (Hanja)[4] and incubated for 24 h at 42°C. The broths were then streaked out onto modified lysine iron agar[5] and brilliant green sulfa agar[3] and incubated for 24 h at 35°C. Suspect colonies were picked and triple sugar iron[3] and lysine iron agar[3] slants were stabbed and incubated for 24 h at 35°C. Poly O[3] and Poly H[6] agglutination tests were used to confirm presumptive positives.

Statistical Analysis

Salmonellae incidence data were analyzed using the chi-squared test for independence.

Chiller bag and tank water pH data were analyzed using the GLM procedure of SAS (SAS Institute, 1998). Sources of variation were treatment (2), first and second chilling steps (2), replication within trial (5), and trial (4), and the mean square error was the error test statistic.

RESULTS AND DISCUSSION

Trials 1 and 2

For trials 1 and 2, TSP significantly reduced the recovery of salmonellae on the day of processing and after 7 d of refrigerated storage when compared with nontreated control carcasses (Table 1). There were no significant differences in the prevalence of salmonellae recovered within the control or TSP-treatment groups from the day of processing to after 7 d of storage at 2°C.

[2]Model MBW-16, Stork-Gamco, Inc., Gainesville, GA.
[3]Difco Laboratories, Detroit, MI.
[4]Becton Dickinson, Sparks, MD.
[5]Oxoid Ltd., Basingstoke, UK.
[6]Microgen, Camberley, Surrey, UK.

TABLE 1. Recovery of salmonellae from trisodium phosphate-treated (TSP) and control carcasses on the day of processing and after 7 d of storage at 2°C for trials 1 and 2 (positive carcasses/number of carcasses sampled)

	Day of processing			After 7 d of storage		
	Control	TSP	*P*	Control	TSP	*P*
Trial 1	9/10	3/10	0.0001	6/10	4/10	0.0308
Trial 2	8/10	6/10	0.1138	9/10	3/10	0.0001
Total	17/20	9/20	0.0001	15/20	7/20	0.0001
Percentage	85	45		75	35	

The high incidence of recovery for the control group (85%) raised concerns about the potential for cross-contamination in the immersion chill tanks. The high incidence, compared with commercial processing, may be attributed to the lack of added chlorine in the chill water (typically 20 ppm). After chilling, the chill water pH in the TSP-treated chiller was 9.4, whereas the control chiller water pH was 7.0, despite an overflow rate of 1 L/min. Higher pH values may have independently influenced salmonellae recovery from the TSP-treated carcasses.

Trials 3 through 7

After modifying the chilling methods to prevent cross-contamination, there was still a significant decrease in salmonellae recovery from TSP-treated carcasses on the day of processing and after 7 d of storage at 2°C compared with controls (Table 2), with some exceptions. However, unlike trials 1 and 2, the recovery of salmonellae was lower after 7 d of storage for both the TSP-treated and control carcasses, $P = 0.0004$ and $P = 0.0002$, respectively.

Fabrizio et al. (2002) reported similar results, where *Salmonella* populations were significantly reduced on half carcasses immediately following TSP treatment and after 7 d of storage at 4°C (reductions of 0.9 and 2.17 $\log_{10}$ cfu/mL, respectively). Also, the residual effect of TSP was demonstrated with a minimal but significant reduction (0.19 $\log_{10}$ cfu/mL) of *Salmonella* from sampling immediately following treatment to after 7 d of storage at 2°C.

The reduction in *Salmonella* incidence may have been due to TSP not being rinsed off or neutralized immediately following treatment. In a recent study by Simmons et al. (2003), salmonellae incidence was reported to be 33.9% for broiler carcasses obtained at the retail level. However, it was not known whether these carcasses were treated with TSP during processing. It was speculated that one possibility for this apparent increase in salmonellae recovery, compared with the FSIS baseline (USDA–FSIS, 1996) (20% in the plant after chilling), may have been injured bacteria recovering during the time between processing and sale to the consumer. In the present study there was no such increase; in fact, a decrease in salmonellae recovery was detected after 7 d of storage at 2°C for both control and TSP-treated carcasses.

Prevalence of salmonellae between trials was highly variable (Table 2). Control groups ranged from 1 to 10/10 positive carcasses and TSP-treated groups ranged from 0 to 5/10 positive carcasses on the day of processing. After 7 d of storage at 2°C, control groups varied from 0 to 5/10 positive carcasses and TSP-treated groups, 0 to 1/10 positive carcasses. Trial 4 was the only trial in which there were more positive carcasses for the TSP-treated group (3/10) than the control group (1/10); in trial 6 there was only one positive carcass for the control and TSP groups combined. After 7 d of storage at 2°C, salmonellae were not recovered from the TSP-treated groups in 3 of the trials. Overall, on 6 of 14 sampling days (2 sampling days for each of 7 trials), salmonellae recovery was not significantly different between TSP-treated and control carcasses. This variation in salmonellae recovery between trials is likely due to variation in flock salmonellae levels when entering the plant.

Chill Water pH

Chill water pH in trials 4 through 7 are presented in Table 3. The largest difference among individual chill

TABLE 2. Recovery of salmonellae from trisodium phosphate-treated (TSP) and control carcasses on the day of processing and after 7 d of storage at 2°C for trials 3 through 7 (positive carcasses/number of carcasses sampled)

	Day of processing			After 7 d of storage		
Trial	Control	TSP	*P*	Control	TSP	*P*
3	8/10	3/10	0.0001	1/10	1/10	1.0000
4	1/10	3/10	0.0350	3/10	0/10	0.0384
5	10/10	5/10	0.0001	5/10	1/10	0.0114
6	1/10	0/10	0.2919	0/10	0/10	1.0000
7	3/10	2/10	0.4901	1/10	0/10	0.2919
Total	23/50	13/50	0.0045	10/50	2/50	0.0047
Percentage	46	26		20	4	

TABLE 3. Carcass bag chill water pH values for trials 4 through 7 for control and trisodium phosphate (TSP) treatment

Treatment	Trial	First chiller[1]	Second chiller[2]
Control	4	7.94	7.43
	5	7.80	7.38
	6	8.00	7.59
	7	7.98	7.68
	Average	$7.93^{A,Z}$	$7.52^{B,Z}$
TSP	4	9.07	8.16
	5	8.96	8.24
	6	9.05	8.32
	7	8.83	8.28
	Average	$8.98^{A,Y}$	$8.25^{B,Y}$

[A,B]Values within a row with no common superscripts differ significantly ($P < 0.0001$).

[Y,Z]Values within a column with no common superscripts differ significantly ($P < 0.0001$).

[1]After first 20-min chill for individually bagged carcasses.

[2]After second 20-min chill for individually bagged carcasses.

water pH was 1.3 pH units for TSP carcasses and 1.9 for control carcasses. There were no significant treatment × trial interactions, so data were combined. Chill water pH was significantly lower after the second chill step compared with after the first chill step for control and TSP-treated carcasses. Although each carcass was thoroughly rinsed off with water 4 times and 20× PBS was added to each chiller bag in the first chill to help buffer pH, the pH readings for the TSP treatment group were still significantly higher than the control group for both the first and second chill (1.05 and 0.73 pH units, respectively).

Due to the higher bag chiller water pH in the TSP treatment, it is uncertain whether the reduction of salmonellae recovery was due exclusively to the TSP treatment or whether 40 min at this elevated pH may have also reduced recovery. Further experiments are necessary to differentiate the direct effects of TSP from secondary, long-term effects of high pH.

This study determined the efficacy of TSP on the day of processing and after 7 d of storage at 2°C. On day of processing and after 7 d of storage, TSP decreased salmonellae recovery compared with the control treatment. After 7 d of storage at 2°C, salmonellae recovery decreased for both the control and TSP-treated carcasses.

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